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Food Chemistry 100 (2007) 1409–1418
Food Chemistry
www.elsevier.com/locate/foodchem

# Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants

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Received 8 August 2005; accepted 29 November 2005

**Abstract**

Ethanolic extracts from various parts of 26 Thai indigenous plants were examined for phenolic constituents and free radical scavenging capacity, to determine their potential as a source of natural antioxidants. Total phenolic content and total flavonoid content were evaluated according to the Folin-Ciocalteu procedure, and a colorimetric method, respectively. The results showed that total phenolic compounds and flavonoid content were higher in seed extracts of berries used in wine production, while the levels in extracts obtained from herbs and vegetables were lower. Chewing plants which have an astringent taste gave a significantly higher total phenolic content and flavonoid content. Antiradical activity determined from $1/EC_{50}$ by the DPPH radical-scavenging method was highest in wine production seeds and chewing plants. The correlation coefficient from regression analysis showed a positive relationship between total phenolic and total flavonoid content ($r = 0.9$). The results suggest that ethanolic extracts of some Thai indigenous plants exhibit a potential for use as natural antioxidants.


*Keywords:* Thai indigenous plants; DPPH; Antioxidant activity; Total phenolic content; Total flavonoid content

## 1. Introduction

There is a growing interest in the problem of lipid oxidation because of its significance for food deterioration. Lipid oxidation is a complex free radical chain process involving a variety of radicals. Oxidation is influenced by temperature, light, air, physical and chemical properties of the substrates, and the presence of oxidation catalysts or initiators. The use of antioxidants in lipid-containing foods is one method to minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase the shelf life of food products (Jadhav, Nimbalkar, Kulkarni, & Madhavi, 1995). An antioxidant is any substance which is capable of delaying, retarding or preventing the development of rancidity or other off-flavour, due to oxidation at a low concentration, compared with that of the oxidizing substrate (Gordon, 2001a). Since antioxidants can be classified according to their protective properties at different stages of the oxidation process and since they act by different mechanisms, they are divided into two main types of antioxidants: primary and secondary antioxidants. Primary antioxidants can inhibit or retard oxidation by scavenging free radicals by donation of hydrogen atoms or electrons, which converts them to more stable products. Secondary antioxidants function by many mechanisms, including binding of metal ions, scavenging oxygen, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen (Gordon, 1990, 2001b).

Since consumers are concerned about the use of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butyl hydroquinone (TBHQ) and propyl, octyl, and dodecyl gallates in lipid-containing foods, there is an interest in developing

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doi:10.1016/j.foodchem.2005.11.032

natural antioxidants from plants. Plants contain a variety of substances called "phytochemicals" (Pratt, 1992), that owe to naturally-occurring minor components present in plants (Caragay, 1992). Flavonoids and other classes of phenolic compounds are important phytochemicals (Johnson, 2001) and tocopherols, carotenoids, and ascorbic acids are also important (Meyers, Watkins, Pritts, & Liu, 2003). Flavonoids are very effective antioxidants (Yanishlieva-Maslarova, 2001). Flavonoids are a large group of naturally-occurring plant phenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones. Flavonoids contain a characteristic $C_6$-$C_3$-$C_6$ structure, with free hydroxyl groups attached to aromatic rings, and they inhibit lipid oxidation by scavenging radicals or by other mechanisms such as singlet oxygen quenching, metal chelation, and lipoxygenase inhibition (Yanishlieva-Maslarova, 2001).

Many plant phenolic compounds exhibiting antioxidant properties have been studied and proposed for protection against oxidation (Pokorny, 2001). Extracts from plants which contribute health benefits to consumers, arising from protection from free radical-mediated deteriorations, and which cause retardation of lipid oxidation (Oktay, Güloin, & Küfrevioğlu, 2003; Van der Sluis, Dekker, Skrede, & Jongen, 2002) had stronger antioxidant activity than that of synthetic antioxidants. For example, in sunflower oil, corn oil and olive oil, some extracts of spices, such as ginger, nutmeg and licorice exhibited longer induction times than those of BHA and BHT evaluated by the oil stability index using the Rancimat (Murcia et al., 2004). Extracts from green tea and grape seeds also showed higher DPPH radical-scavenging activity than that of BHA (Parejo et al., 2002) while sweet grass extracts were similar to BHT in their antioxidant activity, assessed by the change in peroxide value of rapeseed oil oxidized in an oven test (Bandoniené, Pukalskas, Venskutonis, & Gruzdiené, 2000).

An enormous variety of plants has been studied for new sources of phenolic compounds but there are only a few reports about phenolic content and antioxidant activity of extracts from Thai indigenous plants (Chanwitheesuk, Teerawutgulrag, & Rakariayatham, 2004; Laupattarakasem, Houghton, Hoult, & Itharat, 2003). Various parts of many Thai indigenous plants are used as food, beverage, medicine, or for chewing and these are a potential source of new natural antioxidants. Therefore, the objectives of this work were to study a wide range of Thai indigenous plants and plant parts by evaluating the total phenolic content, total flavonoids and free radical-scavenging capacity of ethanolic extracts. The correlations between total phenolic content, total flavonoid content and free radical-scavenging capacity, presented as antiradical activity ($1/EC_{50}$) were also investigated. These investigations are important for gaining more information about the potential natural antioxidants from various parts of Thai plants, for further application of natural plants as antioxidants in food product development.

## 2. Materials and methods

### 2.1. Chemicals

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, hexamethyltetramine, aluminium chloride and rutin were purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA). Gallic acid was purchased from Acros Organics (Morris Plains, NJ, USA). The other chemicals and solvents used in this experiment were analytical grade, purchased from Sigma–Aldrich Co., Ltd (Steinheim, Germany).

### 2.2. Preparation of plant extracts

Twenty-six plant materials of various varieties and plant parts, classified into three groups as berries and fruits, herbs and vegetables, and chewing plants (Table 1), were selected for study. One batch of each plant material was obtained (at least 30 kg) from a wholesale market in March to June 2002 except for the seeds of fruits and berries, which were obtained from food processing factories as by-products. The plant parts included fruit, fruit peel, fruit flesh, seed, seed skin, bud, flower, leaf, as well as tree parts such as stem core and bark. The moisture content of fresh plant material was determined according to AOAC (1995). The collected plant samples were frozen immediately after arrival and stored at −30 °C until extraction time, with less than 2 months storage. From preliminary tests, these extracts from frozen samples gave a free radical-scavenging capacity and total phenolic content comparable to those of extracts prepared from fresh plants. The frozen plants were ground in a blender for 1 min. Ground sample (60 g) was mixed with 95% ethanol (300 ml) in the dark at 25 °C for 4.5 h and shaken during the extraction time to ensure complete extraction (method modified from Velioglu, Massa, Gao, & Oomah, 1998). The extracts were filtered through Whatman No. 4 paper and centrifuged (15 min, 1500*g*). Ethanol was evaporated from the supernatants on a rotary evaporator at 50 mm Hg pressure and 50 °C. The evaporated plant extracts were thick and viscous materials and were kept in air-tight amber bottles after flushing with nitrogen gas for 30 s (Azizah, Nik Ruslawati, & Tee, 1999) and stored in freezer at −20 °C until they were analyzed. Extraction was repeated on a fresh batch of plant material until at least 50 g of extract was collected for each plant material.

### 2.3. Determination of plant extract yield

The yield of evaporated dried extracts based on dry weight basis was calculated from Eq. (1) shown below:

$$\text{Yield } (\%) = (W_1 \times 100)/W_2, \quad (1)$$

where $W_1$ was the weight of extract after evaporation of ethanol and $W_2$ was the dry weight of the fresh plant sample.

Table 1
Moisture content and yield of ethanolic extracts obtained from various plant parts of Thai indigenous plants with scientific name and common name[a]

| Scientific name | Common name | Plant part | Moisture content (%) | Yield (%, db)[b] |
|---|---|---|---|---|
| Berries and fruits | | | | |
| *Antidesma velutinum* Tulas. | | Seed | 38.4 ± 0.0 | 0.5 ± 0.0 |
| *Cleistocalyx operculatus* var. *paniala* (Roxb.) | | Seed | 55.1 ± 0.0 | 0.3 ± 0.0 |
| *Diospyros kaki* L. | Persimmon | Whole fruit | 92.4 ± 0.1 | 3.2 ± 0.1 |
| | | Fruit peel | 86.4 ± 0.1 | 5.3 ± 0.0 |
| | | Fruit flesh | 94.4 ± 0.1 | 3.2 ± 0.1 |
| *Eugenia siamensis* Craib. | Jambolan Plum | Seed | 50.3 ± 0.0 | 0.8 ± 0.0 |
| *Garcinia mangostana* Linn. | Mangosteen | Fruit peel | 62.5 ± 0.0 | 11.8 ± 0.0 |
| *Leucaena glauca* Benth. | Leadtree | Seed | 76.5 ± 0.0 | 0.4 ± 0.0 |
| *Mangifera indica* Linn. | Mango | Seed | 52.3 ± 0.1 | 3.2 ± 0.0 |
| *Nephelium lappaceum* Linn. | Rambutan | Fruit peel | 71.3 ± 0.0 | 1.7 ± 0.0 |
| | | Seed | 36.3 ± 0.2 | 3.7 ± 0.0 |
| *Nephelium hypoleucum* Kurz | Pulasan | Whole fruit | 76.2 ± 0.0 | 1.4 ± 0.0 |
| *Piper nigrum* Linn. | Pepper | Seed | 61.0 ± 0.5 | 0.6 ± 0.0 |
| *Spondias pinnata* Kurz | Hog plum | Fruit flesh | 76.4 ± 0.1 | 2.3 ± 0.0 |
| | | Seed | 52.3 ± 0.1 | 1.2 ± 0.0 |
| *Tamarindus indica* Linn. | Tamarind | Seed | 49.5 ± 0.1 | 3.0 ± 0.0 |
| | | Seed skin | 12.3 ± 0.0 | 0.5 ± 0.0 |
| Herbs and vegetables | | | | |
| *Basella alba* Linn. | Ceylon spinach | Bud | 91.9 ± 0.1 | 0.8 ± 0.0 |
| *Careya sphaerica* Roxb. | Tummy wood | Bud | 75.4 ± 0.0 | 2.3 ± 0.0 |
| *Cratoxylum formosum* Dyer. | | Bud | 79.8 ± 0.1 | 4.1 ± 0.1 |
| *Hydrocharis dubia (Bl.)* Back. | Frogs bit | Bud | 95.0 ± 0.0 | 0.8 ± 0.0 |
| *Hydrolea zeylanica* (L.) Vahl. | | Bud | 83.6 ± 0.1 | 1.1 ± 0.0 |
| *Lasia spinosa* (Linn.) Thw. | | Bud | 94.2 ± 0.3 | 1.4 ± 0.1 |
| *Leucaena glauca* Benth. | Leadtree | Bud | 49.9 ± 0.1 | 3.0 ± 0.0 |
| *Limnocharis flava* Buch. | | Bud and flower | 94.7 ± 0.3 | 1.9 ± 0.0 |
| *Momordica charantia* Linn. | Balsum pear | Bud and leaf | 87.0 ± 0.0 | 1.3 ± 0.0 |
| *Sesbania grandiflora* Desv. | Cork wood | Flower | 91.1 ± 0.1 | 2.6 ± 0.0 |
| *Spondias pinnata* Kurz | Hog plum | Bud | 88.4 ± 0.3 | 0.2 ± 0.0 |
| *Syzygium gratum* (Wight) S.N.Mitra var. gratum | | Bud | 90.4 ± 0.1 | 1.2 ± 0.0 |
| Chewing plants | | | | |
| *Acacia catechu* (L.F.) Willd. | Black catechu | Bark | 16.3 ± 0.1 | 0.9 ± 0.0 |
| *Areca catechu* Linn. | Betel nut | Whole fruit | 90.2 ± 0.0 | 0.2 ± 0.0 |
| | | Kernel | 91.2 ± 0.1 | 0.3 ± 0.0 |
| *Cassia fistula* Linn. | Golden shower | Stem core | 11.4 ± 0.0 | 1.0 ± 0.0 |
| *Piper betel* Linn. | Betel leaf | Leaf | 82.6 ± 0.4 | 1.5 ± 0.0 |

[a] Values are the mean ± standard deviation ($n = 3$).
[b] Dry weight basis of the original sample of plant parts.

### 2.4. Determination of total phenolic content

The total phenolic content of the ethanolic extracts was determined using the Folin-Ciocalteu reagent (Kähkonen et al., 1999). Each evaporated thick and viscous extract (~0.8 to 0.9 g ± 0.01 mg) was diluted with 5 ml methanol. The sample of each plant extract solution (200 μl) was transferred into a test tube and then mixed thoroughly with 1 ml of Folin-Ciocalteu reagent. After mixing for 3 min, 0.8 ml of 7.5% (w/v) sodium carbonate was added. The mixtures were agitated with a vortex mixer, then allowed to stand for a further 30 min in the dark, and centrifuged at 3300*g* for 5 min. The absorbance of plant extracts and a prepared blank were measured at 765 nm using a spectrophotometer (UV–vis model 1601, Shimadzu, Kyoto, Japan). The concentration of total phenolic compounds in all plant extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight of plant, using the linear Eq. (3) derived from Eq. (2), which was determined from known concentrations of gallic acid standard prepared similarly. Data were reported as a mean ± standard deviation for three replications

$$\text{Absorbance (at 765 nm)} = \text{constant} \times (\text{gallic acid concentration}) \quad (2)$$

$$\text{Gallic acid equivalents} = \text{Absorbance} \times (\text{at 765 nm})/0.0508 \quad (3)$$

### 2.5. Determination of total flavonoid content

The total flavonoid content of plant extracts was evaluated by a colorimetric assay according to the method of Bonvehí, Torrent, and Lorente (2001). One millilitre of 0.5% (w/v) hexamethyl tetramine, 20 ml of acetone, and 2 ml of 0.1 M HCl were added to each finely ground thawed-frozen plant sample (5 g) and boiled under reflux

for 30 min. The resulting solution was filtered through Whatman paper No. 4 and the residue was further washed with 20 ml of acetone. The filtrate volume was finally adjusted to 100 ml with acetone. Ten millilitres of filtrate from each plant extract was pipetted into a separating funnel, along with 20 ml of $H_2O$ and then the aqueous phase was extracted with 25 ml of ethyl acetate. Further extraction with 25 ml ethyl acetate was carried out at least twice. The extraction was repeated twice using 50 ml of $H_2O$ each time. The total amount of extract in the ethyl acetate layer collected from the separating funnel was subsequently made up to 100 ml with ethyl acetate. To determine the total flavonoid content, 10 ml of extract in ethyl acetate was pipetted into a test tube and mixed with 1 ml of 2% (w/w) $AlCl_3$ in methanol solution containing 5% acetic acid, using a vortex mixer. The absorbance was read immediately at 425 nm using a spectrophotometer (UV–vis model 1601, Shimadzu, Kyoto, Japan). The absorbance of a prepared blank was also recorded. Total flavonoid content expressed as rutin equivalents in milligrams per gram dry weight of plant was also determined using the linear Eq. (4) from standard curve of rutin standard. Data were reported as mean ± standard deviation for three replications

$$\text{Rutin equivalents (RE)} = \text{Absorbance (at 425 nm)}/13.33 \quad (4)$$

### 2.6. Determination of free radical-scavenging using DPPH

The free radical-scavenging activity of plant extracts was evaluated using the stable radical DPPH, according to the method of Masuda et al. (1999). A series of extract concentrations with different ratios of extracts to methanol, i.e. 1:10, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$, were prepared. Then, 4.9 ml of each diluted plant extract was mixed with 100 μl of 5 mM DPPH in methanol. The mixtures of different extract concentrations and DPPH were placed in the dark at 37 °C for 30 min. The absorbance of each sample of plant extract containing DPPH ($A_1$) was read at 517 nm using a spectrophotometer (UV–vis model 1601, Shimadzu, Kyoto, Japan). The absorbance of each sample of plant extract dilution without DPPH ($A_s$), and only DPPH solution without plant extract ($A_o$, called control) were also recorded, to determine the DPPH radical-scavenging activity (modifying the method of Tachibana, Kikuzaki, Lajis, & Nakatani, 2001). All determinations were performed in triplicate. The percentage of DPPH radical-scavenging activity of each plant extract determined at these seven concentrations within the range of dose-response (at least 10–90% reduction in absorbance) was calculated as shown:

$$\text{DPPH radical scavenging activity (\%)} = [A_o - (A_1 - A_s)]/A_o \times 100, \quad (5)$$

where $A_o$ is the absorbance of the control solution (containing only DPPH), $A_1$ is the absorbance in the presence of the plant extract in DPPH solution and $A_s$, which is used for error correction arising from unequal colour of the sample solutions, is the absorbance of the sample extract solution without DPPH.

The percentage of DPPH radical-scavenging activity was plotted against the plant extract concentration (μg/ml) to determine the amount of extract necessary to decrease DPPH radical concentration by 50% (called $EC_{50}$). The $EC_{50}$ value of each extract was estimated by sigmoid non-linear regression using SigmaPlot 2000 Demo (SPSS Inc., Chicago, IL, USA). The unit of $EC_{50}$ was later converted to μg/μg DPPH. These values were changed to antiradical activity ($A_{AR}$) defined as $1/EC_{50}$: the higher the antioxidant activity, the higher the value of the antiradical activity.

### 2.7. Statistical analysis

Each of the measurements described above was carried out in at least three replicate experiments, and the results are reported as the mean and standard deviation.

## 3. Results and discussion

Since the ethanolic extracts from 26 Thai indigenous plants were obtained from various varieties and plant parts (fruit, peel, flesh, seed, seed skin, bud, leaf, flower, stem core, bark), we classified the plants into three groups: (1) berries and fruits, (2) herbs and vegetables and (3) chewing plants, for characterizing the selected plant parts in terms of the total phenolic compounds, total flavonoids and free radical-scavenging activity. For leadtree and hog plum, we used both parts of the berries or fruits and bud. Therefore, each part of both plants was analyzed and classified according to the parts that were used within the groups of berries and fruits, and herbs and vegetables. Seed is defined as plant part containing the embryo for the group of berries and fruits. In the case of tamarind, the seed skin refers to a thin brown skin layer covering the seed. For the herbs and vegetables, the bud includes outgrowths on a stem or branch, consisting of a shortened stem and immature leaves. The buds as a plant part were selected for study because the buds of vegetables and herbs are consumed frequently in Thailand. The group of chewing plants was selected for studies of the antioxidant activity because the highly astringent taste reflects a high phenolic content (Llaudy et al., 2004). Betel nut, in the group of chewing plants called seed for chewing, is one of the dark red seeds (kernels) of the betel palm that is wrapped in betel leaves with lime and chewed by some people in Asia. Some people use the whole fruit of the betel nut for chewing but others use only the kernel. The difference between the whole fruit and kernel is that the kernel is obtained by removal of a fibrous husk surrounding the kernel.

Differences in polarity (and thus different extractability) of the antioxidative components are obviously the reason why extraction yields and antioxidant activity of the

extracts differ (Julkunen-Tiito, 1985; Marinova & Yanishlieva, 1997). Ethanol was used as extraction medium in this work because it is the most widely-used solvent and safe to apply in foods.

### 3.1. The yield of ethanolic extracts

Among the 26 plant extracts studied, the moisture content of the fresh plant depends on the plant group and the type of plant part. The yield of the ethanolic extract from all groups of plants was calculated based on a dry weight basis, in order to eliminate the influence of the different moisture contents of the plants.

For most berries and fruits, the moisture content of the whole fruit or fruit flesh was higher than 90% but it was lower in the fruit peel and seed (ranging from 36% to 86%). The tamarind seed skin showed the lowest moisture content (12.3%) in this group, due to roasting being applied for 15 min during sample preparation, prior to extraction, in order to separate the thin layer of skin from the tissue inside the seed. The extract yields of fruit seeds, such as rambutan (3.7%), mango (3.2%) and tamarind (3.0%), were higher than those obtained from berry seeds (0.3–1.2%). The higher yield from fruit seeds may be due to extraction of the carbohydrate component in the seed, as reported for mango kernel seed by Kabuki et al. (2000). The extract yields from other fruit parts, such as peel and flesh, were 1.7–11.8%.

For the group of herbs and vegetables, the moisture content of the plants varied from 75% to 95%, except for the bud of leadtree (50%), due to the characteristic of this plant having a low moisture content. Yields of extracts from this herb and vegetable ranged from 0.2% to 4.1%. Considering the chewing plants, the moisture content depends on the plant part: leaf and whole fruit or bark. The moisture content of fruit and leaf were high (80–91%) while those of bark and stem core were low (11–16%). However, the yields of ethanolic extracts from these chewing plants were lower than 1.5%.

### 3.2. Total phenolic content and total flavonoid content in the studied plant tissues

Phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorny, 2001). Flavonoids are phenolic compounds, which are very effective antioxidants (Yanishlieva-Maslarova, 2001). The Folin-Ciocalteu method is a rapid and widely-used assay, to investigate the total phenolic content but it is known that different phenolic compounds have different responses in the Folin-Ciocalteu method (Kähkonen et al., 1999). Therefore, in this work, we calculated the total phenolic contents in units of mg gallic acid equivalent of phenolic compound as shown in Table 2. The total phenolic content differed among the different types and parts of plants and each plant extract contained a lower total flavonoid content than the total phenolic content, since other compounds besides flavonoids are phenolic substances in plants (Pietta, 2000).

For the group of berries and fruits, seeds from *Antidesma velutinum* Tulas., *Cleistocalyx operculatus* var. *paniala* (Roxb.) and *Eugenia siamensis* Craib. were obtained as by-products of wine production in Thailand. The total phenolic content of these seeds was very high (123–180 mg GAE/g dry weight of plant extract) compared to that obtained from other fruit and berry seeds (20–54 mg GAE/g dry weight of plant extract). The reason for the lower total phenolic content of fruit seeds may be the contribution of carbohydrates in the extracts (data not shown). The total phenolic content of fruit peel ranged from 13 to 42 mg GAE/g dry weight of plant extract. In the case of tamarind, seed skin gave a high total phenolic content (134 mg GAE/g dry weight of plant extracts). The total flavonoid content of the berry and fruit group was low, compared to the total phenolic content (Table 2). The total flavonoid content of seeds of *Antidesma velutinum* Tulas., *Cleistocalyx operculatus* var. *paniala* (Roxb.) and *Eugenia siamensis* Craib. was remarkably high and was the highest in the group of berries and fruits (44–50 mg RE/g dry weight of plant extracts) compared to those obtained from other plant seeds (5–23 mg RE/g dry weight of plant extract). However, extracts with higher phenolic content did not always have a higher flavonoid content, as was evident for the seed of *Antidesma velutinum* Tulas. which had a higher total flavonoid content (50 mg RE/g dry weight of plant extract) compared with that of *Cleistocalyx operculatus* var. *paniala* Roxb. (44 mg RE/g dry weight of plant extract), although the total phenolic content was lower (123 and 174 mg GAE/g dry weight of plant extract, respectively). The results suggest that different plant extracts contain different levels of total flavonoids as a proportion of the total phenolic compounds. The total flavonoids of fruit peel ranged from 2 to 10 RE/g dry weight of plant extract. The skin layer of tamarind also gave a high value of total flavonoid content (41 RE/g dry weight of plant extracts).

The most selected and studied plant part in herbs and vegetables was the bud, which comprises the outgrowth on a stem or branch consisting of a shortened stem and immature leaves. Based on total phenolic content in the extracts from herbs and vegetables, the selected parts can be divided into three ranges of GAE values. The lower, middle and higher ranges of total phenolic compounds were below 10, 10–20 and higher than 40 mg GAE/g dry weight of plant extract, respectively. Plants with the lower total phenolic content included the buds of *Hydrolea zeylanica* (L.) Vahl., *Lasia spinosa* (Linn.) Thw., and *Limnocharis flava* Buch. (5–7 mg GAE/g dry weight of plant extract), which are mostly vegetables consumed fresh with chilli paste in Thailand and most commonly found in rice fields as weeds. Plant extracts from herbs and vegetables with a higher phenolic content also contained a higher fla-

Table 2
Total phenolic content, total flavonoid content and DPPH radical-scavenging activity of Thai indigenous plants[a]

| Scientific name | Plant part | Total phenolics (mg GAE/g dw)[b] | Total flavonoids (mg RE/g dw)[b] | DPPH radical-scavenging activity ($EC_{50}$, μg/μg DPPH)[c] |
|---|---|---|---|---|
| Berries and fruits | | | | |
| *Antidesma velutinum* Tulas. | Seed | 123.3 ± 0.3 | 50.3 ± 0.0 | 0.07 ± 0.01 |
| *Cleistocalyx operculatus* var. *paniala* (Roxb.) | Seed | 173.6 ± 1.9 | 44.2 ± 0.2 | 0.09 ± 0.00 |
| *Diospyros kaki* L. | Whole fruit | 17.8 ± 0.7 | 2.5 ± 0.0 | 0.98 ± 0.00 |
| | Fruit peel | 12.9 ± 1.2 | 1.6 ± 0.0 | 1.70 ± 0.01 |
| | Fruit flesh | 22.8 ± 1.6 | 4.1 ± 0.1 | 0.58 ± 0.00 |
| *Eugenia siamensis* Craib. | Seed | 180.5 ± 1.3 | 50.4 ± 0.3 | 0.15 ± 0.02 |
| *Garcinia mangostana* Linn. | Fruit peel | 24.9 ± 0.7 | 10.9 ± 0.1 | 1.09 ± 0.00 |
| *Leucaena glauca* Benth. | Seed | 20.4 ± 0.0 | 5.3 ± 0.0 | 7.01 ± 0.09 |
| *Mangifera indica* Linn. | Seed | 51.6 ± 0.1 | 14.6 ± 0.1 | 0.34 ± 0.01 |
| *Nephelium lappaceum* Linn. | Fruit peel | 42.3 ± 0.1 | 9.6 ± 0.0 | 1.46 ± 0.04 |
| | Seed | 43.5 ± 0.4 | 13.3 ± 0.1 | 0.46 ± 0.00 |
| *Nephelium hypoleucum* Kurz | Whole fruit | 89.6 ± 0.2 | 11.1 ± 0.7 | 0.40 ± 0.00 |
| *Piper nigrum* Linn. | Seed | 53.1 ± 0.4 | 22.8 ± 0.1 | 0.33 ± 0.00 |
| *Spondias pinnata* Kurz | Fruit flesh | 47.2 ± 0.2 | 12.7 ± 0.1 | 0.62 ± 0.00 |
| | Seed | 50.7 ± 0.1 | 17.8 ± 0.3 | 0.43 ± 0.00 |
| *Tamarindus indica* Linn. | Seed | 40.7 ± 0.1 | 23.2 ± 0.1 | 0.49 ± 0.01 |
| | Seed skin | 134.4 ± 0.1 | 41.3 ± 0.1 | 0.14 ± 0.00 |
| Herbs and vegetables | | | | |
| *Basella alba* Linn. | Bud | 15.5 ± 0.1 | 6.2 ± 0.0 | 1.48 ± 0.00 |
| *Careya sphaerica* Roxb. | Bud | 54.5 ± 0.3 | 20.5 ± 0.1 | 0.43 ± 0.04 |
| *Cratoxylum formosum* Dyer. | Bud | 63.4 ± 0.5 | 25.5 ± 0.1 | 0.23 ± 0.00 |
| *Hydrocharis dubia (Bl.)* Back. | Bud | 20.4 ± 0.2 | 8.9 ± 0.0 | 0.82 ± 0.00 |
| *Hydrolea zeylanica* (L.) Vahl. | Bud | 7.4 ± 0.0 | 3.6 ± 0.0 | 6.14 ± 0.05 |
| *Lasia spinosa* Thw. | Bud | 6.4 ± 0.1 | 4.4 ± 0.1 | 7.49 ± 0.02 |
| *Leucaena glauca* Benth. | Bud | 52.2 ± 1.6 | 22.3 ± 0.0 | 0.68 ± 0.01 |
| *Limnocharis flava* Buch. | Bud and flower | 5.4 ± 0.1 | 3.7 ± 0.2 | 7.42 ± 0.04 |
| *Momordica charantia* Linn. | Bud and leaf | 50.9 ± 0.9 | 21.6 ± 0.1 | 0.59 ± 0.00 |
| *Sesbania grandiflora* Desv. | Flower | 50.6 ± 0.6 | 13.1 ± 0.1 | 0.58 ± 0.00 |
| *Spondias pinnata* Kurz | Bud | 42.6 ± 0.2 | 14.8 ± 0.1 | 1.48 ± 0.02 |
| *Syzygium gratum* (Wight) S.N.Mitra var.gratum | Bud | 57.3 ± 0.1 | 23.6 ± 0.1 | 0.55 ± 0.00 |
| Chewing plants | | | | |
| *Acacia catechu* (L.F.) Willd. | Bark | 177.7 ± 0.2 | 41.8 ± 0.2 | 0.05 ± 0.00 |
| *Areca catechu* Linn. | Whole fruit | 52.5 ± 0.2 | 12.6 ± 0.1 | 0.47 ± 0.00 |
| | Kernel | 137.3 ± 0.3 | 42.8 ± 0.1 | 0.18 ± 0.00 |
| *Cassia fistula* Linn. | Stem core | 103.6 ± 0.2 | 25.4 ± 0.2 | 0.16 ± 0.01 |
| *Piper betel* Linn. | Leaf | 57.5 ± 0.6 | 14.9 ± 0.1 | 0.32 ± 0.00 |

[a] Values are the mean ± standard deviation ($n = 3$).
[b] Dry weight basis of the original sample of plant parts.
[c] Calculated by using dry weight of the ethanolic plant extract.

vonoid content (13–26 mg RE/g dry weight of plant extract).

The phenolic content of selected chewing plant extracts was higher than that in extracts from some studied herb and vegetable extracts (53–178 mg GAE/g dry weight of plant extracts). In addition, the flavonoid content of extracts from chewing plants was in the range 13–43 RE/g dry weight of plant extract. However, since these plant parts are used as chewing materials but without being swallowed, the toxicity of extracts from this group should be further investigated before they are used in food products.

We can observe that there are five plant buds in the group of herbs and vegetables containing remarkably high phenolic contents (51–63 mg GAE/g dry weight of plant extract) and flavonoid contents (20–26 mg RE/g dry weight of plant extract): *Careya sphaerica* Roxb., *Cratoxylum formosum* Dyer., *Leucaena glauca* Benth., *Momordica charantia* Linn. *and Syzygium gratum* (Wight) S.N. Mitra var. gratum. These plants may be considered suitable for further investigation of their potential antioxidant activity in foods because usually they can be consumed fresh without toxicity.

### 3.3. Free radical-scavenging activity of plant extracts using DPPH

Since the main mechanism of antioxidant action in foods is radical scavenging, many methods have been developed in which the antioxidant activity is evaluated by the scavenging of synthetic radicals in polar organic solvents such as methanol at room temperature. The radicals used include 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals (Gordon, 2001a). In this study, the DPPH

method was selected to evaluate the antioxidant activity of plant extracts because it is one of the most effective methods for evaluating the concentration of radical-scavenging materials active by a chain-breaking mechanism (Niki, 1987). The DPPH radical is a stable free radical and the DPPH radical-scavenging activity was determined by the decrease in absorbance at 517 nm, due to reduction by the antioxidant (AH) or reaction with a radical species, as shown in the Eqs. (6) and (7) (Gordon, 2001a)

$$DPPH^{\bullet} + AH \rightarrow DPPH - H + A^{\bullet} \quad (6)$$

$$DPPH^{\bullet} + R^{\bullet} \rightarrow DPPH - R \quad (7)$$

The DPPH radical-scavenging capacity in the studies was reported after 30 min reaction time for each diluted plant extract. All the plots of the plant extracts showed sigmoid non-linear curves (data not shown). $EC_{50}$ value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period, is a parameter widely used to measure antioxidant activity; a smaller $EC_{50}$ value corresponds to a higher antioxidant activity of the plant extract. The $EC_{50}$ value of various plant extracts shown in Table 2 was determined based on scavenging activity per unit mass of DPPH in μg.

For the group of berries and fruits, the DPPH radical-scavenging activity ($EC_{50}$) values of ethanolic extracts of seeds from *Antidesma velutinum* Tulas., *Cleistocalyx operculatus* var. *paniala* (Roxb.) and *Eugenia siamensis* Craib., containing notably high levels of both phenolics and flavonoids, were found to be very low (about 0.07–0.15 μg/μg DPPH), compared to those obtained from other seed extracts (0.33–0.49 μg/μg DPPH), except that of *Leucaena glauca* Benth., which was 7.0 μg/μg DPPH (Table 2). The antiradical activity ($A_{AR}$) defined as $1/EC_{50}$ was plotted for extracts from various berries and fruits as shown in Fig. 1. As expected, extracts with higher antiradical activity were obtained from the three seed extracts mentioned above, as well as from extracts from the tamarind seed skin (0.14 μg/μg DPPH) (Table 2).

As discussed previously, in the group of herbs and spices, samples with a low total phenolic content, namely the buds of *Hydrolea zeylanica* (L.) Vahl., *Lasia spinosa* (Linn.) Thw., and *Limnocharis flava* Buch. (5–7 mg GAE/g dry weight of plant extracts), also exhibited a low total flavonoid content, and a high $EC_{50}$ value (higher than 6 μg/μg DPPH), as shown in Table 2, indicating a very low antiradical activity (Fig. 2). Extracts from other herbs and vegetables also appeared to have lower antiradical activity, compared with the activity of extracts from the seeds. Amongst the herbs and vegetables, the highest antiradical activity was found in the extract of *Cratoxylum formosum* Dyer. (bud) which can be easily found in North-East Thailand. However, this antiradical activity was still lower than that of extracts of seeds obtained from wine production and tamarind seed skin. Similar findings, that seed extracts exhibited a much higher antioxidant activity than those from the edible portions, was also reported by Soong and Barlow (2004).

In chewing plants, the $EC_{50}$ value of bark extract from *Acacia catechu* (L.F.) Willd. was lowest (0.05 μg/μg DPPH) and gave the highest antiradical activity among all studied plant parts (Fig. 3). The highest antiradical activity of bark extract ( *Acacia catechu* (L.F.) Willd.) reflects the high phenolic content (178 mg GAE/g dry weight of plant extract)

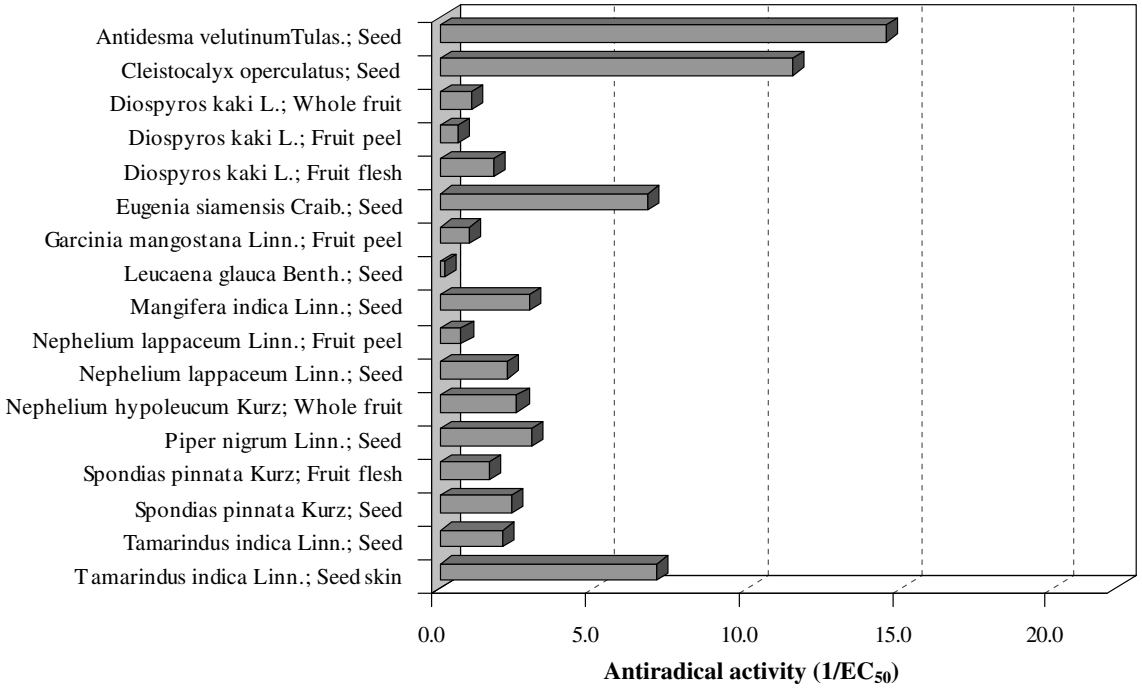


Fig. 1. Antiradical activity ($1/EC_{50}$) of ethanolic extracts from various plant parts of berries and fruits.

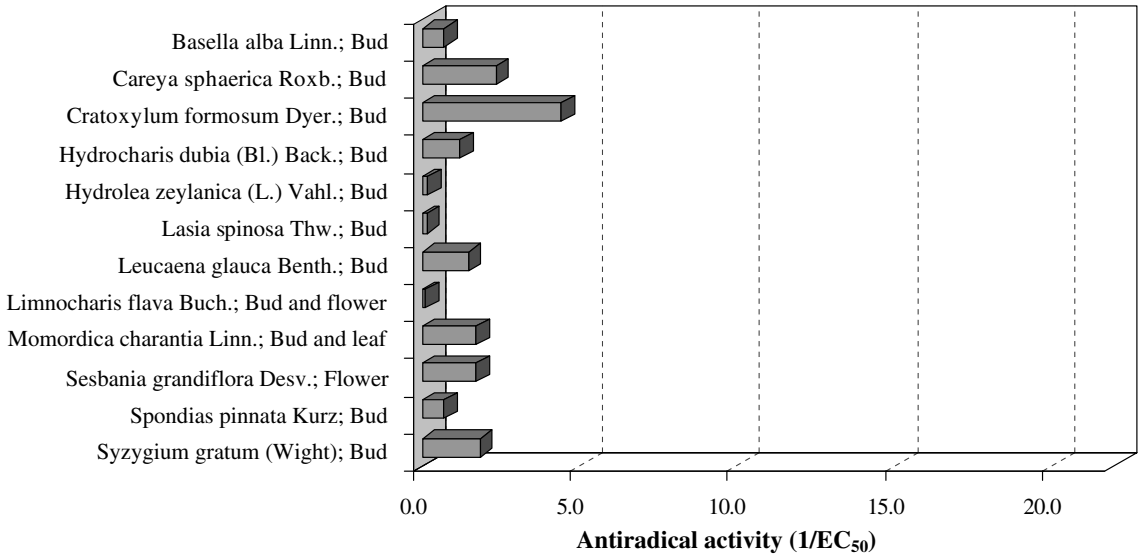


Fig. 2. Antiradical activity ($1/EC_{50}$) of ethanolic extracts from bud, leaf and flower of herbs and vegetables.

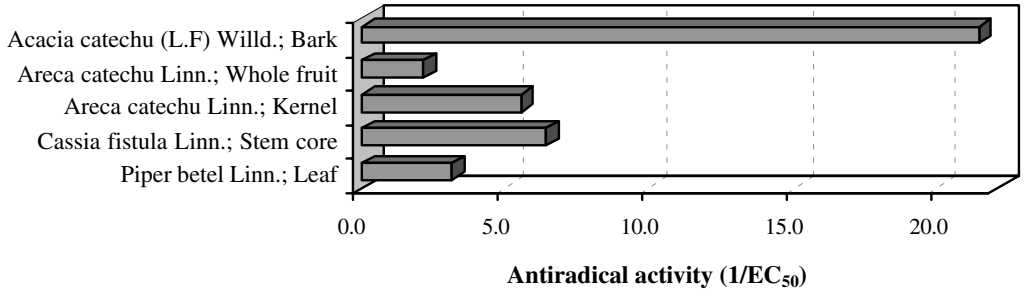


Fig. 3. Antiradical activity ($1/EC_{50}$) of ethanolic extracts from plant parts of chewing plants.

and flavonoid content (42 mg RE/g dry weight of plant extract) (Table 2). However, it should be noted that the seed extract from *Eugenia siamensis* Craib. had both the highest phenolic content (180 mg GAE/g dry weight of plant extract) and flavonoid content (50 mg RE/g dry weight of plant extract) among all the extracts investigated, but the antiradical activity was lower than that of the bark extract from *Acacia catechu* (L.F.) Willd. When considering the extracts of *Areca catechu* Linn. (kernel) and *Cassia fistula* Linn. (stem core) in the group of chewing plants, the extracts appeared to contain high antioxidant activity ($EC_{50}$ values = 0.18 and 0.16 μg/μg DPPH, respectively) with high phenolic content (137 and 104 mg GAE/g dry weight of plant extracts) and flavonoid content (43 and 25 mg RE/g dry weight of plant extracts), respectively (Table 2).

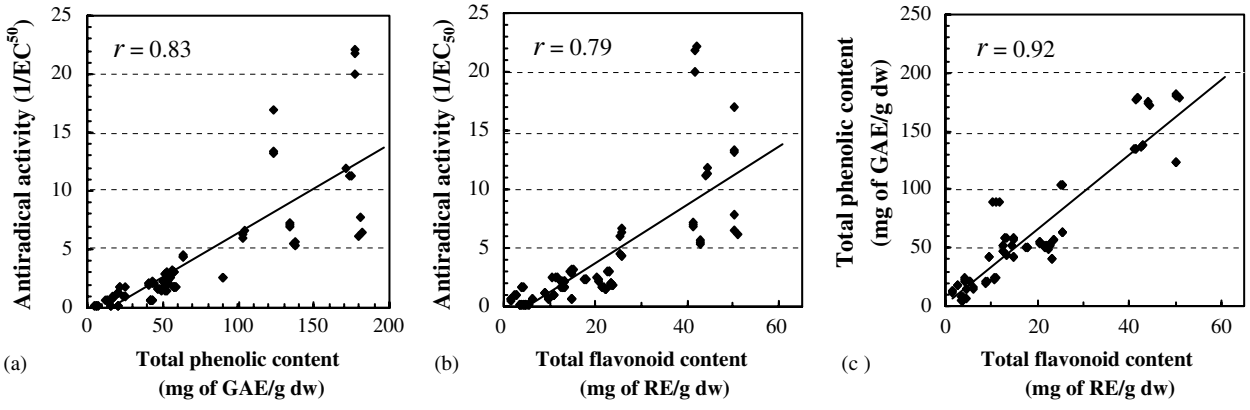


Fig. 4. Correlation between total phenolic content and antiradical activity (a), total flavonoid content and antiradical activity (b), and total phenolic content and total flavonoid content (c) of ethanolic extracts from Thai indigeneous plants.

### 3.4. Correlation between total phenolic and flavonoid content and antiradical activity

It is interesting to observe the correlation between the phenolic content and antioxidant activity between the plant extracts, since phenolic compounds contribute directly to antioxidant activity (Duh, 1999). In this study, there was a distinct correlation between studied parameters (total phenolic content, total flavonoid content and antiradical activity) in selected Thai indigenous plant parts. The antiradical activity ($1/EC_{50}$), as a function of total phenolic content and total flavonoid content is shown in Figs. 4(a) and (b), respectively, and correlation coefficients ($r$) of those plots, calculated from linear regression analysis, were about 0.8 for all ethanolic extracts of plant parts. However, the literature includes studies reporting a weak correlation between antioxidant activity and total phenolics (Kähkonen, Hopia, & Heinonen, 2001; Velioglu et al., 1998). When the relationship between total phenolic content and total flavonoid content of all ethanolic extracts was plotted as shown in Fig. 4(c), the correlation coefficient ($r$) between these two parameters was higher than 0.9 indicating that there is a significant positive relationship between the total phenolic and flavonoid contents of all plant extracts selected in this study.

## 4. Conclusion

According to the evaluation of phenolic constituents and free radical-scavenging capacity from ethanolic extracts from various parts of 26 Thai indigenous plants, the extracts of berries used in wine production were found to have a higher antiradical activity than those obtained from herbs and vegetables, whereas chewing plants with an astringent taste had a high level of total phenolic content and flavonoid content. The correlation coefficients exhibited a high positive relationship between total phenolic and flavonoid contents in the plant extracts and antiradical activity. The present study suggests that ethanolic extracts of these Thai indigenous plants are a potential source of natural antioxidants. However, the toxicity of the plant extracts with high antioxidant activity should be tested, to confirm their safety for use as food additives. In addition, the characteristics of the phytochemicals and the antioxidant mechanisms of the extracts should be further studied, to gain more understanding of their antioxidant activity in food systems.

## Acknowledgments

This material is based upon work supported by a grant from Kasetsart University Research and Development Institute (KURDI) and by Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant no. PHD/0224/2546). This research was partially supported by a Grant-in-Aid for Thesis and Dissertation Support Fund from the Graduate School at Kasetsart University. We also thank Dr. Michael H. Gordon from the University of Reading for helpful suggestions and corrections.

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